

World-wide whale worms? A new species of *Osedax* from the shallow north Atlantic

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We describe a new species of the remarkable whalebone-eating siboglinid worm genus, *Osedax*, from a whale carcass in the shallow north Atlantic, west of Sweden. Previously only recorded from deep-sea (1500–3000 m) whale-falls in the northeast Pacific, this is the first species of *Osedax* known from a shelf-depth whale-fall, and the first from the Atlantic Ocean. The new species, *Osedax mucofloris* sp. n., is abundant on the bones of an experimentally implanted Minke whale carcass (*Balaenoptera acutorostrata*) at 125 m depth in the shallow North Sea. *O. mucofloris* can be cultured on bones maintained in aquaria. The presence of *O. mucofloris* in the shallow North Sea and northeast Pacific suggests global distribution on whale-falls for the *Osedax* clade. Molecular evidence from mitochondrial cytochrome oxidase 1 (CO1) and 18S rRNA sequences suggests that *O. mucofloris* has high dispersal rates, and provides support for the idea of whale-falls acting as ‘stepping-stones’ for the global dispersal of siboglinid annelids over ecological and evolutionary time.

Keywords: Annelida; Polychaeta; Siboglinidae; Pogonophora; CO1

1. INTRODUCTION

Whale-falls represent one of the more extraordinary and poorly sampled habitats on the planet (Smith & Baco 2003; Dahlgren *et al.* 2004; Rouse *et al.* 2004). To date, all published *in situ* studies of whale carcasses on the seafloor have been restricted to the deep-sea (900–3000 m) of the northeast Pacific; these investigations have revealed a species-rich ‘whale-fall fauna’ with similarities to hydrothermal vents and cold seeps (Smith & Baco 2003). The sulphide and lipid-rich nature of decomposing whale-bones is thought to create a habitat intermediate to that of vents and seeps, and to have allowed vent/whale conspecifics such as vesicomyid clams (Baco *et al.* 1999; Distel *et al.* 2000) and polynoid polychaetes (Glover *et al.* *in press*) to use whale-falls as dispersal ‘stepping-stones’ over evolutionary or ecological time-scales.

The recent discovery of the whale-fall specialist *Osedax*, a novel annelid clade with unusual reproductive biology (e.g. male dwarfism) and an apparently new form of bacterial endosymbiosis for the animal kingdom, highlights our poor knowledge of deep-sea ecosystems (Rouse *et al.* 2004). Surprisingly, shallow-water whale falls are even less well studied, and have so far been restricted to some descriptions of mollusc species associated with bones accidentally trawled by fishermen (Marshall 1900; Warén 1989), and no *in situ* experimental studies have previously been carried out. For this reason, in October 2003, we sank the remains of a stranded Minke whale in the Kosterfjord, Swedish west coast, in 125 m of water,

and in August 2004 recovered vertebrae from the whale-fall that were covered with large numbers of a novel siboglinid annelid, *Osedax mucofloris* sp. n. (figure 1).

2. MATERIAL AND METHODS

(a) Sample collection and morphological analysis

A 5.3 m carcass of a female Minke whale, *Balaenoptera acutorostrata*, was implanted at 58° 53.1' N; 11° 06.4' E, 125 m depth, Kosterfjord, Sweden in October 2003 (Dahlgren *et al.* unpublished data). Measurements of bottom water temperature indicated only small variations during the year of 4.8–7.5 °C, with salinity 34.3–34.7 PSu, and oxygen levels of 4.7–6.3 ml l⁻¹. The whale carcass was located in a region of regularly trawled soft sediment. Using Phantom XL Remotely Operated Vehicles (ROVs) with forward-mounted sampling scoops (the small ROVs lack manipulator arms), we recovered vertebrae with *O. mucofloris* populations in August 2004, January 2005 and March 2005. Bones were recovered by driving the sampling-scoop of the ROVs into the sediment surrounding the bone and capturing them in the netting. The recovered bones were kept in filtered seawater and transferred to aquaria in the laboratory with through-flowing filtered seawater at 8 °C. Observations of live worms were undertaken on bones in the aquaria, and individual specimens excavated and preserved for either morphological or molecular analysis.

Specimens for morphological analysis were preserved in 10% formalin in seawater or 2% glutaraldehyde in sodium cacodylate buffer; 8 µm thin paraffin-wax sections were made and stained in Mayer's haemalum and eosin (Cooper 1988). Specimens for scanning electron microscopy were dehydrated in ethanol, critical point dried, gold-coated and imaged using a Phillips XL-30 SEM.

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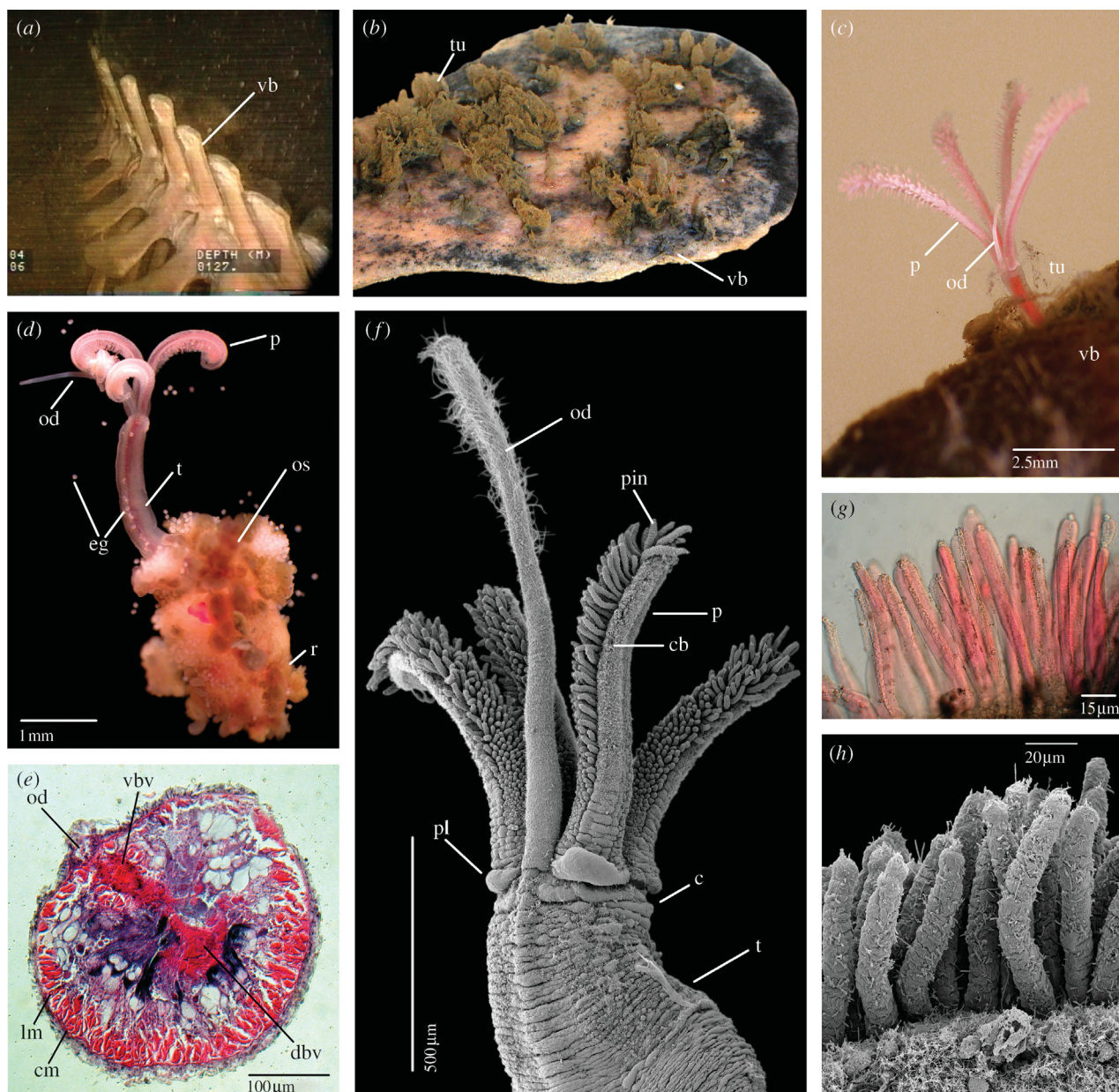


Figure 1. *Osedax mucofloris*. (a) Video still of skeletonized Minke whale carcass at 125 m depth, Kosterfjord. (b) Vertebral process with individuals of *O. mucofloris* retracted into mucous tubes. (c) Photomicrograph of live specimen emergent from bone, with palps and oviduct extended. (d) Photomicrograph of live, dissected specimen, with trunk, ovisac and root system. (e) Transverse section of anterior trunk. (f) Scanning electron micrograph of trunk and palps. (g) Photomicrograph of pinnules in live specimen. (h) Scanning electron micrograph of pinnules with ciliary bands and epibiotic bacteria. c, collar; cb, ciliary band; cm, circular muscle; dbv, dorsal blood vessel; eg, eggs; lm, longitudinal muscle; od, oviduct; os, ovisac; p, palps; pin, pinnules; pl, plaque; r, root; t, trunk; tu, tube; vb, vertebrae; vbv, ventral blood vessel.

(b) Molecular genetic analysis

Individual female worms ($n=18$) were excavated from bone tissue, the mucous tubes removed and genomic DNA extracted with DNeasy Tissue kit (Qiagen) following the manufacturers instructions. An alignment of siboglinid annelids was used to design a pair of primers (OsCO1f 5' aattattcgaattgaattagg 3' and OsCO1r 5' aatcaaataggtgttggaatag 3') for PCR amplification of a 606 bp fragment of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene using standard PCR conditions (Palumbi 1996). Direct sequencing was performed with CEQ dye terminator chemistry and separated on a Beckman CEQ capillary sequencer. All CO1 fragments were sequenced in both directions using the amplification primers (OsCO1f and OsCO1r) and assembled and proofread with aid of the

program SEQMAN (DNASTAR, Inc.). The data was aligned with MEGALIGN (DNASTAR, Inc.) and examined with MACCLADE 4.07 (Maddison & Maddison 2000). For distance calculations and parsimony analyses we used the program PAUP* (Swofford 2002). Sequence data for the corresponding CO1 fragment of *Osedax rubiplumus* ($n=6$) and *Osedax frankpressi* ($n=19$) was retrieved from GenBank (AY586486–AY586510) and reanalysed using the same methods (table 1). Haplotype connectedness was illustrated by drawing a parsimony network (Avise *et al.* 1979).

Using the same methods as described above, universal primers (Halanych *et al.* 1998) for amplification and sequencing were applied to determine a 1751 bp fragment of the 18S rRNA gene (GenBank accession number AY941263). Outgroup choice followed the most recent

Table 1. Three species of *Osedax*. Frequency distribution and GenBank Accession numbers of CO1 haplotypes.

species	haplotype	frequency	GenBank
<i>O. mucofloris</i> sp. n.	A	5	AY827562 ^a
	B	7	AY827563 ^a
	C	2	AY827564 ^a
	D	1	AY827565 ^a
	E	1	AY827566 ^a
	F	1	AY827567 ^a
	G	1	AY827568 ^a
<i>O. frankpressi</i>	A	10	AY586486
	B	2	AY586490
	C	1	AY586491
	D	1	AY586492
	E	1	AY586494
	F	1	AY586496
	G	1	AY586502
	H	1	AY586503
	I	1	AY586504
<i>O. rubiplumus</i>	A	3	AY586505
	B	2	AY586507
	C	1	AY586509

^a Sequence determined in this study.

study of siboglinid relationships (Rousset *et al.* 2004). All siboglinid taxa for which the 18S rRNA sequence has been determined were used in the analysis (table 2). The data was aligned using CLUSTAL W (Thompson *et al.* 1994) and ambiguous positions excluded. Bayesian statistic analyses was conducted with MRBAYES v3.0B4 (Huelsenbeck & Ronquist 2001) and a GTR evolutionary model chosen using MODELTEST 3.6 (Posada & Crandall 1998). Parsimony and bootstrap analyses were conducted with PAUP* (Swofford 2002).

3. RESULTS

(a) Observations of live *Osedax mucofloris* sp. n.

Live specimens of *O. mucofloris* were not observed during the original ROV video surveys, and we confirmed the presence of *O. mucofloris* only after detailed aquaria-based observations (figure 1c). During later ROV operations, it was possible to see *O. mucofloris* on the bones *in situ*, usually the palps, projecting above a thin layer of bacterial mat and sediment. Disturbance created by the ROV and bone-recovery caused the animals to retract wholly into the bone. When placed in aquaria, with clean, chilled seawater, the animals would emerge from the bone and be clearly visible to the naked eye. Over a period of several minutes, the worms would first extend the palps, and then the oviduct (see electronic supplementary material—video). Any disturbance to the aquarium tank would result in the animals immediately withdrawing into the bone.

(b) Systematics

(i) Taxonomy

Annelida Lamarck, 1809, Canalpalpata Rouse & Fauchald, 1997, Siboglinidae Caullery, 1914. *Osedax* Rouse *et al.*, 2004

(ii) Material examined

Osedax mucofloris sp. n. Type material. Kosterfjord, Skagerrak, western Sweden, ROV dives from Tjärnö

Table 2. Taxa used in the analysis of 18S rRNA.

taxon name	GenBank
<i>Osedax frankpressi</i>	AY577885
<i>Osedax rubiplumus</i>	AY577894
<i>Osedax mucofloris</i> sp. n.	AY941263 ^a
<i>Galathealinum brachiosum</i>	AF168738
<i>Polybrachia</i> sp.	AF168739
<i>Spirobrachia</i> sp.	AF168740
<i>Escarpi</i> spicata	AF168741
<i>Lamellibrachia barhami</i>	AF168742
<i>Oasisia alvinae</i>	AF168743
<i>Ridgeia piscesae</i>	AF168744
<i>Riftia pachyptila</i>	AF168745
<i>Tevnia jerichonana</i>	AF168746
<i>Siboglinum fiordicum</i>	AF315060
<i>Sclerolinum brattstromi</i>	AF315061
<i>Siboglinum ekmani</i>	AF315062
<i>Owenia</i> sp.	AY611447
<i>Myriochele</i> sp.	AY340437

^a Sequence determined in this study.

Marine Laboratory vessel R/V *Lophelia* 30 August 2004 (58° 53.1' N; 11° 06.4' E), 125 m depth; holotype, mature adult female, (NHM 2005.239, Natural History Museum, London) collected from vertebrae of 5.3 m Minke whale carcass; paratypes, two females, (NHM 2005.240–241) two females, Los Angeles County Museum (LACM-AHF POLY).

(iii) Diagnosis

Holotype, live specimen visible as four white to pink palps emergent from bone surface, palps 5–6 mm in length, surrounded at the base by thin, mucous tube (figure 1a–c). Oviduct white, extending one-third length of uncontracted palps (figure 1c). Palps of equal length 0.8 mm (contracted), with numerous pinnules of length 0.1 mm (contracted), ciliary band running entire length of each palp at base of pinnules (figure 1d,e,f,h). Pinnules width of 0.01 mm, densely packed and white to pink in live specimens (figure 1g), with microvilli of length 0.05 mm. Trunk region 6–8 mm in length, 0.5 mm in width, partially embedded within bone matrix (figure 1d), composed principally of bands of longitudinal muscles, glands and major dorsal and ventral blood vessels; mouth and gut absent (figure 1d,e). Ventral plaques on collar (peristomial region) of trunk (figure 1f). On dissected specimens, oviduct visible running into ovisac and root structure, containing numerous eggs in trunk region (figure 1d). Vascularized root system 2–10 mm in length, burrowing a shallow depression of maximum depth 2–3 mm in bone matrix in a branched, mycelial form (figure 1d). Numerous (greater than 100) eggs (diameter 85–90 µm) released from ovisac on disturbance (figure 1d). Chaetae and opisthosomal region not observed. Epibiotic rod-shaped bacteria (length 1–1.5 µm, width 300 nm) present over surface of trunk, palps and pinnules, absent from roots (figure 1f,h).

(iv) Etymology

From Latin *muco*, mucous and *floris*, flower, being in reference to the flower-like nature of the animal on emergence from a mucus lined tube, and the presence of plant-like 'roots' that are embedded within the whale bone.

(v) *Remarks*

Specimens of *O. mucofloris* are morphologically closer to *O. frankpressi* (Rouse *et al.* 2004), sharing a similar gelatinous tube and short, lobulate roots. However, they differ from the two northeast Pacific species in the colour of their palps (variable white to pink as opposed to blood-red or white-striped), the colour of their root systems (yellow as opposed to green), their small size at maturity, and by genetic distances of 18–23% (CO1 sequences) and 1.4–4.2% (18S rRNA). *Osedax mucofloris* also differ from *O. rubiplumus* (Rouse *et al.* 2004) in the shape of the root system (short, lobulate roots as opposed to long, branching roots) and the nature of their tubes (gelatinous mucous tubes rather than rigid cylindrical tubes).

(c) *Population genetics and reproductive biology*

Within the *O. mucofloris* sample ($n=18$) we detected seven unique haplotypes of 507 bp observing no indels or stop codons. Three and nine haplotypes were found in the GenBank sequences for *O. rubiplumus* ($n=6$) and *O. frankpressi* ($n=19$) respectively. GenBank accession numbers and observed haplotype frequencies are presented in table 1. A parsimony network (*sensu* Avise *et al.* 1979) illustrates the differences between the haplotypes of the three species (figure 2b). The highest Kimura-2-parameter corrected genetic pairwise distances between two haplotypes within the *O. mucofloris* sample was 1.2%. Corresponding values for *O. rubiplumus* and *O. frankpressi* CO1 haplotypes were 0.40% and 0.59% respectively (Rouse *et al.* 2004). CO1 genetic distances between the species were close to saturation (*O. mucofloris*–*O. frankpressi* 19.0–20.3% and *O. mucofloris*–*O. rubiplumus* 21.5–22.7%).

All specimens of *O. mucofloris* examined were ripe, egg-producing females and despite detailed observation of live specimens held in aquaria, we find no evidence as yet for the presence of males.

(d) *Phylogenetic position inferred from 18S rRNA sequences*

The final 18S alignment included 1634 unambiguous positions of which 317 sites were parsimony informative, 1213 constant and 104 variable positions parsimony uninformative (alignment is available from <http://www.treebase.org>). Phylogeny analyses using Parsimony Jackknife and Bayesian statistics was congruent in all strongly supported nodes (posterior probability and jackknife values of 100), indicating a monophyletic *Osedax* clade and that *O. mucofloris* is the sister to *O. frankpressi* (figure 2a). Both methods, however, failed to unambiguously resolve the relationship between the three major clades of siboglinid taxa (whale, vent & seep, and sediment).

4. DISCUSSION

The phylogeny of the pogonophoran annelids has been a matter of debate for many decades, but recent studies have shown that all of the taxa formerly known as Vestimentifera or Pogonophora are highly derived polychaetes that fall within the clade Siboglinidae (McHugh 1997; Halanynch *et al.* 2001; Rouse 2001). The phylogeny of the siboglinids points to an evolutionary trend of increasing specialization on sulphide-rich habitats (Halanynch *et al.* 2001; Schulze & Halanynch 2003), from sediment-dwelling frenulates exposed to low sulphide levels (caused by anoxia) through

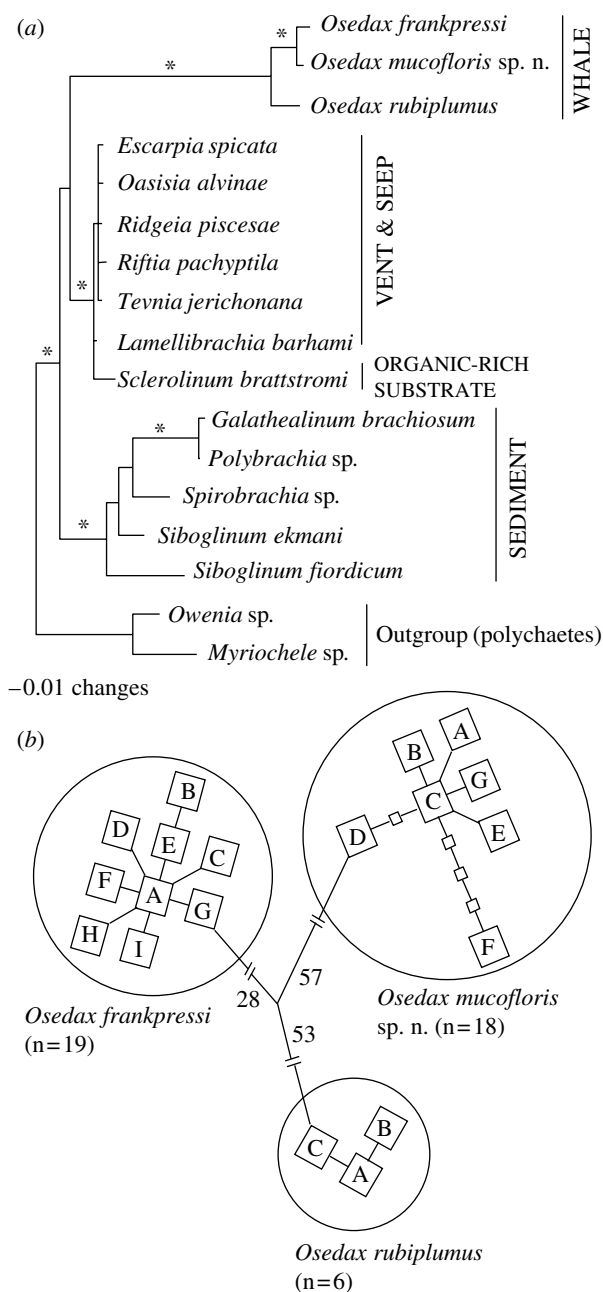


Figure 2. Phylogenetic position and haplotype network for *Osedax mucofloris*. (a) Bayesian analysis of 18S rRNA show that *O. mucofloris* is close to *O. frankpressi* Rouse *et al.*, 2004. Posterior probability, bootstrap and parsimony jackknife values of 99–100 indicated by asterisks. (b) CO1 haplotype network. Sample size given in brackets. Lines connecting haplotypes equivalent to one mutational event if not otherwise stated. Small squares represent inferred haplotypes not found in sample.

to the giant tube worms, such as *Riftia pachyptila*, which live on sulphide-rich hydrothermal vents. This seemingly ordered progression has been complicated by the recent discovery of the whale-fall *Osedax* clade (Rouse *et al.* 2004) and by results presented here. Phylogenetic evidence from 18S rRNA sequences places *O. mucofloris* as sister group to *O. frankpressi* (figure 2a) and the entire *Osedax* clade as a highly derived sister group to the vent/seep vestimentiferans.

The derived nature of *Osedax* worms is supported by morphological evidence—the loss of the opisthosome, chitinous tube and the evolution of a branching ‘root’ system on the posterior portion of the animal. However,

functionally similar root systems have been described from seep vestimentiferans (Freytag *et al.* 2001), and sediment-dwelling lucinid (Taylor & Glover 2000) and thyasirid (Dufour & Felbeck 2003) bivalves, which are used to 'mine' sulphides from hydrocarbon seeps or anoxic sediments. The interiors of decomposing whale bones are rich in sulphides (Deming *et al.* 1997), and we observed *O. mucofloris* growing in regions of blackened, possibly sulphide-rich bone (figure 1b). Whilst the presence of lipid-degenerating bacteria in northeast Pacific *Osedax* has been suggested (Rouse *et al.* 2004), there is no evidence as yet to suggest that these animals are not also using sulphide-based chemoautotrophy; further investigation of the functional anatomy and microbial ecology of the root system is required before we can say with certainty that vestimentiferan roots are non-homologous to the roots of *Osedax*.

The size of the north Atlantic *Osedax* habitat is dependent on the current population densities of cetaceans that are known to create lipid-rich whalebone reefs. Previous experiments with deep-sea implantations of dolphin carcasses report relatively quick consumption of soft tissue by scavengers (Jones *et al.* 1998), however long term data on the fate of skeletal material for these smaller Cetacea was not reported. Of the much larger-boned balaenopterid whales, the most abundant in the north Atlantic is the Minke (*Balaenoptera acutorostrata*) with a current population estimated at 149 000 (IWC 2004). At the Swedish whale-fall site, CO1 sequences from *O. mucofloris* (figure 2b) reveal the presence of seven haplotypes on a single whale bone. This supports an hypothesis of multiple founder events from several source populations or a single multiple-individual founder event from a heterogeneous background community within the North Sea. Detailed examination of at least 50 mature specimens indicated an absence of the dwarfed males reported for northeast Pacific *Osedax* species (Rouse *et al.* 2004), however we observed that *O. mucofloris* appeared able to reproduce and grow to maturity within one month on defaunated bones placed into the aquaria. This is in accord with suggestions of rapid colonization and growth rates for some pogonophoran worms (Lutz *et al.* 1994) and asynchronous reproduction in vestimentifera (Tyler & Young 1999). With more precise estimates of the number of whale-falls in the north Atlantic, the risk of extinction by habitat loss (Smith *in press*) may in the future be modelled using a metapopulation approach (Hanski 1998).

In terms of ocean circulation, the collection sites of the genus *Osedax* are very widely separated. The deep northeast Pacific and shallow northeast Atlantic lie at opposite ends of the global 'conveyor belt' of thermohaline circulation (Broecker 1995), and any exchanges in the wind-driven surface currents would require passage through the gyres of multiple ocean basins. Analysis of DNA sequences indicates that the *Osedax* phylogeny is discordant with its geographic distribution (figure 2a,b). The two northeast Pacific species of *Osedax* are present on the same whale-fall, yet they do not form the sister clade to the Swedish *Osedax* species in the analysis. This evidence suggests that dispersal events, rather than vicariance, have played a stronger role in shaping the current distributions of known *Osedax* species. This is in contrast to the pattern suggested for many obligate hydrothermal vent taxa, where vicariance events (caused by the movement of plates and

spreading centres) have led to strong concordance between phylogeny and geography (e.g. Van Dover *et al.* 2002). One possible dispersal route for shallow-water *Osedax* species would be a trans-arctic invasion between north Pacific and north Atlantic ocean basins, which has been reported for shelf-depth molluscs (Vermeij 1991; Väinölä 2003).

The greater role of dispersal in determining phylogeographic patterns within the *Osedax* clade provides support for the hypothesis of whale-falls acting as 'stepping-stones' (over evolutionary time-scales) for chemosynthetic fauna in the deep sea (Smith *et al.* 1989). Whilst vent and seep habitats are restricted to appropriate geologic settings such as mid-ocean ridges and continental margins, whale-falls may occur anywhere throughout the world's oceans. Recent evidence regarding the longevity of whale bones on the seafloor (Schuller *et al.* 2004) and estimates of pre-whaling population sizes (Roman & Palumbi 2003) suggest that the whale-fall habitat may be much larger than previously envisaged, and was greater still in pre-whaling days (Smith *in press*). The presence of a specialist fauna on such a shallow and relatively small balaenopterid greatly extends the range of the whale-fall habitat in both spatial (Smith & Baco 2003) and geological (Squires *et al.* 1991) scales.

The evidence presented here of a potentially global clade of bone-eating worms, hitherto unknown except from a single deep-sea region, is surprising given that whale bones have been routinely trawled up on the shelf and slope by fishermen, and in some cases examined (after drying) by scientists who did not report *Osedax*-like worms (e.g. Marshall 1900; Marshall 1987). However, the generally small size of these worms, their cloaking in a mucous matrix, their behaviour of withdrawing into the bone when stressed, and their tendency to shrivel into tiny threads upon drying (personal observations) cause these animals to be very easily overlooked. We suggest that careful, microscopic examination of whale bones freshly recovered from the seafloor may reveal that *Osedax* is widespread in the oceans.

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